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MORPHOGENETIC EFFECTS OF SEVERAL PLANT GROWTH REGULATORS (PGR) ON *IN VITRO* DEVELOPMENT OF BINAHONG (*Anredera cordifolia* L.) LEAF

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Abstract

The aim of this study was to observe the effects of several plant growth regulator on the development of Binahong (*Anredera cordifolia* L.) leaf *in vitro*. Binahong is a potential medicinal plant widely used in Indonesia. The leaves used in this research were young leaves collected from the tips of the Binahong vine. The media used was MS medium with several treatments of various plant growth regulators in different concentrations. Both auxins IBA (indole butyric acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) and also cytokinin BAP (benzyl amino purine) were used with concentrations of 0.5-3 ppm added to basic MS media. Fifteen repetitions were done for each treatment. White callus was obtained from MS media added with 1-3 ppm 0f 2,4-D and green callus was obtained from combination of 0.5ppm IBA+0.5ppm BAP, 0.5ppm IBA+1ppm BAP and 1ppm IBA+0.5ppm BAP. A shoot-like callus was induced from the addition of 1 ppm BAP + 0.5 IBA. The growth of roots also occurred on several explants planted on media MS containing both 2,4- and a combination of BAP and IBA. The different result on binahong leaf grown on different treatments of PGR is due to many factors affecting plant morphogenesis *in vitro*. This in effect can be continued for further research as it can lead to the potential of Binahong as an alternative model plant in tissue culture experiments.

Key words : morphogenesis, plant growth regulators, *in vitro*, binahong leaf

INTRODUCTION

Binahong (*Anredera cordifolia* L.) is a potential medicinal plant that is widely used in Indonesia. Almost all parts of the plant can be used such as the stem, root, flower and leaves (Manoi, 2009). Anredera belongs to the Basellaceae family and is also known as Madeira Vine due to its ornamental succulent vine and in China it is known as Dheng Shan ci (Usman, 2010). Besides Indonesia, other nations also consume this plant as vegetables and traditional medicine such as in Taiwan (Chuang *et al*., 2007). The Binahong plant contains bioactive compounds such as flavonoids, saponins, triterpenoids and coumarins (Djamil *et al*., 2012). Extract of Binahong leaves has antibacterial property and was able to stop the growth of *Staphylococcus aureus* and *Pseudomonas aureginosa* (Khunaifi, 2010). Saponin of Binahong was investigated by Astuti *et al*. (2011) and discovered that saponins compound was found on all parts of Binahong plant. Crude saponin substance was found mostly in the tubers and leaves of the plant. Saponin found in Binahong is known as ancordin which is effective when used as herbicide.

From the different compounds found in Binahong and the potential for the use as a cure for different illness, there is a potential research for studies in increasing the content of the compounds and study on the regulation of the compounds synthesis. As in the study of tobacco

cell culture, there was a wide variation on the ability of nicotine synthesis (Furuya *et al*., 1972 *cit.* Ganapathi *et al*., 2004) and different effects of PGR such as 2,4-D and IAA that can affect the production of nicotine in tobacco callus (Takahashi and Yamada, 1973). Study on the extract of catharantine from the *Catharanthus roseus* can also be produced from callus cultures (Pandiangan, 2011). Catharantine is a precursor of vincristine which is used as an anti cancer.

The callus produced in the two examples above, was induced using tissue culture which is a method of plant regeneration used for a wide range of plants and regeneration method. The purpose of plant tissue culture also vary, depending on the type of plant, the media and other factors that can affect plant cell differentiation. As demand increases for natural medicinal products, the development of medicinal plants via tissue culture is currently focused on using plant materials as 'potential factories' for secondary phytochemical products (Anand, 2010). This type of production is also known as 'phytoproduction' and many compounds have been produced from plant tissue culture production method (Linden, 2014). As binahong has the potential to be developed for its medicinal properties and the use of tissue culture has the potential to increase the amount of compounds or secondary metabolites, the first step in the development of Binahong tissue culture is to observe the effects of certain media and plant growth regulators that are often used in plant tissue culture. Besides the advantage of the different compounds found in the Binahong plant, an efficient *in vitro* regeneration system is vital for the development of the plant for biotechnology and breeding purposes (Neelakandan and Wang, 2012).

For the establishment of tissue culture system that can increase the production of secondary metabolites, several strategies must be established for the method of tissue culture, optimization of culture conditions and selection of highly productive clones (Ruffoni *et al*., 2010). Successful establishment of tissue culture system can increase the efficiency of in vitro protocol for the production of the phytochemical product. Other advantage of establishing this system is to obtain the effects of plant growth regulators on the morphogenesis of the plant used in tissue culture. Morphogenetic studies have been done on a various of plants in an *in vitro* environment. The aims of those studies also vary from simple micropropagation, production of certain secondary metabolites from medicinal plants, to detecting the effects of exogenous plant growth regulators (PGR) on clones of transformed plant species (Vanisree *et al.*, 2004 and Koperdakova *et al*., 2009). In micropropagation techniques, the effect of different PGR's are studied to induce direct or indirect organogenesis and all results are analyzed to see the different effects of the PGR. Study on the morphogenetic effect of PGR on medicinal plant is important because some of the medicinal compounds are usually localized in morphologically specialized tissues or organs and can be induced in specific organized cultures or by undifferentiated cell cultures (Vanisree *et a*l., 2004). The aim of this study is to investigate the response of Binahong leaf tissues grown *in vitro* on MS media containing different plant growth regulators and to obtain the right callus for a more effective production of bioactive compounds.

RESEARCH METHOD

The explants used were young binahong leaves obtained from plants growing in the Biology Garden, Faculty of Science and Mathematics, Yogyakarta State University. The leaves were sterilized using detergent, sterile aquadest, 70% alcohol, and solutions of bleachwith concentrations of 15% and 10%. Sterilization was first carried out in a semi-sterile room and continued in the Laminar air Flow (LAF) cabinet where the leaves were cut into 1 x 1 cm pieces before planting it on the culture media. For the sterilization of equipments, brown wrapping paper and aluminium foil were used as well as autoclave for wet sterilization. An oven was used for dry sterilization of pinsets, petridish and scalpels after being autoclaved.

The media used were MS (Murashige and Skoog) media supplemented with 30 g/liter sucrose and 8 g/liter agar and plant growth regulators including 2,4- dichlorophenoxyacetic acid

(2,4-D), indole butyric acid (IBA) and benzyl amino purine (BAP) in different combinations and concentrations. The culture media was adjusted to pH 5.7 before autoclaving.There were six treatments of PGR on the MS media : 1 ppm 2,4-D, 2 ppm 2,4-D, 3 ppm 2,4-D, 0.5ppm IBA+0.5ppm BAP, 0.5ppm IBA+1ppm BAP and 1ppm IBA+0.5ppm BAP. For each treatments there were 5 repetitions of culture media, with each media containing 3 explants, thus there were a total of 15 repetitions. MS media without PGR was used as the control.Explants were kept in the incubation room, under light condition and temperature of 16-20 ^{0}C for 4-8 weeks. Observation was carried out to see the initiation time of the explant's respond in the *in vitro* environment. The respond in the form of callusing, rooting or the growth of shoot was recorded along with photos of the explants.

RESULT AND DISCUSSION

The potential medicinal properties of the binahong plant has been widely studied and there are several compounds that is effective in curing several diseases. Plant tissue culture is an alternative method for producing important medicinal compounds from plants. Large scale production of those compounds must first be investigated in a smaller scale to find the right propagation technique. Important compounds in binahong was investigated and result showed that most of the phytocompounds were found in the leaves and tubers (Astuti et al., 2011; Djamil *et al.*, 2012). In this research, binahong leaves were used as explants materials to obtain a high concentration of the medicinal compounds that is easy to extract.

1. Callusing

Callus first appeared on the binahong leaf explants 3 days after planting on MS media containing 1 ppm 2,4-D. This was the fastest induction time from all of the treatments. The callus formed were all the same type in all of the media added with 2,4-D. Compact watery callus formed from the 3 media in the first week after planting although some callus were white and some were yellow (Figure 1). The longest time of callus initiation was in the 3 ppm 2,4-D media where callus started to grow 11 days after planting. Compared to the control (explants planted on MS media without PGR), the callus induced were different in numbers and type. The type of callus formed on explants planted on MS media was white and grew only on the tip of the leaf vein. The callus was small and only occurred in 20% of the total explants.

The growth of callus in the media without PGR suggests that the endogenous hormones inside the leaf explants were enough to cause callusing after the leaves were wounded/cut during the *in vitro* experiment. The presence of meristematic cells and parenchyma tissues that are made of undetermined cells in the leaves, can cause rapid proliferation (de-differentiation) to produce cell masses known as callus (Mantell *et al*., 1985).

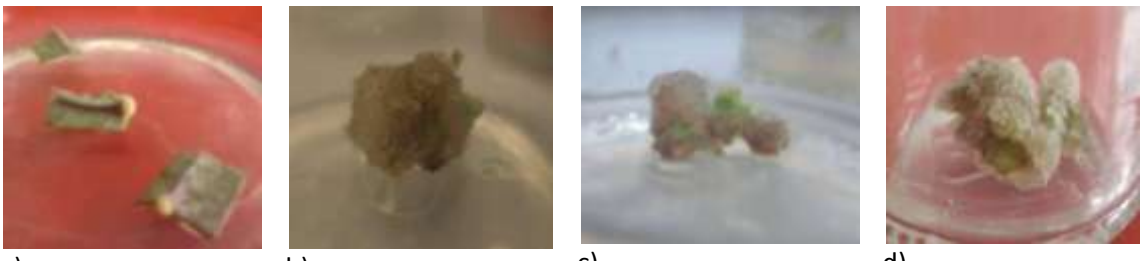

Figure 1. Different types of callus formed on binahong leaf explants 4 weeks after planting on media MS with (a) no PGR, (b) 1 ppm 2,4-D, (c) 2 ppm 2,4-D and (d) 3 ppm 2,4-D.

Addition of the auxin 2,4-D increased the percentage of callusing on the leaf explants. In the media added with 1 ppm 2,4-D all of the explants grew callus that were white, compact and watery. An increase in the concentration of 2,4-D did not increase the percentage of callusing. Only 80% of the explants in media containing 2 ppm and 3 ppm 2,4-D grew callus. The callus

formed in the media with 3 ppm 2,4-D were white and friable. This difference is caused by complex interaction between the type of explants, level of endogeneous hormone, media and tissue culture environment. Friable callus is usually easier to be used in liquid cell cultures while compact callus can be directly extracted for its phytochemicals.

2. Growth of shoot-like callus

The addition of BAP and IBA caused the growth of shoot-like callus. The effect was observed on the edges of the leaves where the tissues were wounded during the cutting of the explants. Wounding of plant tissues can trigger the growth of callus but in this case, with the addition of BAP and IBA in the media, caused the growth of shoot-like callus. The callus looked like shoots because of the green colour and the shape of the callus that did not resemble compact or friable types such as seen in the media with 2,4-D.

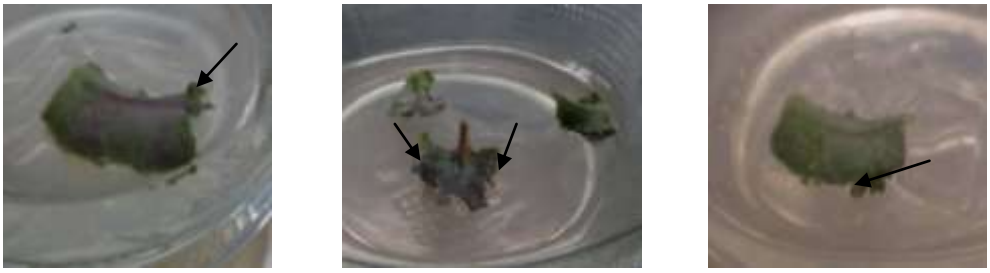

Figure 2. Different types of shoot-resembling callus 6 weeks after planting on media MS with (a) 0.5 ppm BAP+0.5 ppm IBA, (b) 0.5 ppm BAP+1 ppm IBA, and (c) 1 ppm BAP+0.5 ppm IBA (note : the black arrows point at the callus)

Only a small number of the explants grew shoot-like callus, approximately 20 % and also in the 3 types of media. The media that cause the growth of shoot-like callus contained BAP and IBA in almost the same concentrations. The almost balanced concentrations between the two PGR caused the cells in the explants to differentiate producing shoot-like structure that in the long term became de-differentiated into callus. This change can be caused by the endogenous hormones in the explants which could not support the growth of the shoots in the presence of low concentrations of cytokinin in the media.

Figure 3. Shoot-like callus observed using 3-dimensional microscope with 4 different optical zoom

In figure 3, the shoot-like callus was observed using a 3 dimensional microscope and the shape of callus was seen to be round, compact, green and with a few friable yellow callus which increased over time. The green shoot-like callus showed that the cells still produced chloroplast but eventually disappeared as the tissue turned more callus-like. The callus grew compact and yellow and with sub-culture the callus turned brown due to the release of phenol which is found in the leaves of Binahong.

3. Growth of root

On several explants, growth of roots were observed. Usually, the addition of auxins IBA or IAA in the media can induce rooting *in vitro* (Koperdakova *et al*., 2009 ; Daffalla *et al*., 2011). However in this experiment rootings were observed on explants grown on media containing 2,4-D and combination of BAP and IBA (figure 4). All of those media usually induce callus as can be seen from photos in figure 2 and 3. The growth of roots in several explants can be explained from the point where roots grew on the explants. On all of the observed rootings, it occurred on explants cut from the base of the leaf thus containing a part of the leaf petiole. On this part of the leaf, the content of endogeneous hormone may differ from other parts of the leaf. A high content of endogeneous auxin can support the growth of roots from this part of the Binahong leaf. Conventionally, Binahong plants are cultivated by cuttings or planting of the tubers. Thus it is normal to see the growth of roots from the point where the plants are usually cut and used for cuttings. On the Binahong plant, tubers also form usually near the leaf petiole. As interaction can occur between endogenous hormone and the PGR in the media, rooting occurred even though in the presence of callus inducing PGR such as 2,4-D. This shows that the source of explant can strongly affect morphogenetic respond of a plant in an *in vitro* environment.

Figure 4. Rooting on several Binahong explants 6 weeks after planting on MS media with (a) 1 ppm 2,4-D, (b) 3 ppm 2,4-D, (c) 0.5 ppm BAP + 1 ppm IBA and (d) 1 ppm BAP + 0.5 ppm IBA.

CONCLUSION AND SUGGESTION

The development of plant tissue culture for the production of phytochemicals from potential medicinal plants are currently in progress, other benefits from this study can also be used in morphogenetic study of plant development. In this research, the effect of plant growth regulators (2,4-D, IBA and BAP) on the development of Binahong leaf can cause the growth of callus, shoot-like callus and roots. The growth of callus occurred on media MS without PGR and with 2,4-D (1-3 ppm), while shoot-like callus grew on media containing a combination of BAP and IBA. Rooting occurred on explants that contained part of the leaf petioles. The different respond may be due to interaction between endogenous hormone and external PGR contained in the media. This morphogenetic respond also confirm that explant source and plant growth regulators can affect the development of pant *in vitro*. Further research on the underlying process behind morphogenesis in Binahong can be done to generate a more efficient tissue culture protocol in producing useful phytochemicals.

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